(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 September 2003 (04.09.2003)

PCT

(10) International Publication Number
WO 2003/072014 A3

(51) International Patent Classification[7]: C12N 1/00

(21) International Application Number:
PCT/US2002/016877

(22) International Filing Date: 28 May 2002 (28.05.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/359,843	25 February 2002 (25.02.2002)	US
10/154,951	24 May 2002 (24.05.2002)	US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:

US	10/154,951 (CIP)
Filed on	24 May 2002 (24.05.2002)
US	60/359,843 (CIP)
Filed on	25 February 2002 (25.02.2002)

(71) Applicant *(for all designated States except US)*: **MPEX BIOSCIENCE, INC.** [US/US]; 5500 Campanile Drive, San Diego, CA 92182-4614 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SABBADINI, Roger, A.** [US/US]; 12509 Del Sol Road, Lakeside, CA 92040 (US). **SURBER, Mark** [US/US]; Apt. A, 344 "G" Avenue, Coronado, CA 92118 (US). **BERKLEY, Neil** [US/US]; 3226 Erie Street, San Diego, CA 92117 (US). **SEGALL, Anca** [US/US]; 1911 33rd Street, San Diego, CA 92102 (US). **KLEPPER, Robert** [US/US]; 12828 War Horse Street, San Diego, CA 92129 (US). **GIACALONE, Matthew** [US/US]; 4121 Udall Street #2, San Diego, CA 92107 (US). **GERHART, William** [US/US]; 4324 Resmar Road, La Mesa, CA 91941 (US).

(74) Agent: **HUNT, Dale, C.**; Knobbe, Martens, Olson & Bear, LLP, 14th Floor, 2040 Main Street, Irvine, CA 92614 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *with amended claims*

(88) Date of publication of the international search report:
12 February 2004

Date of publication of the amended claims: 22 April 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MINICELL COMPOSITIONS AND METHODS

(57) Abstract: The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

WO 2003/072014 A3

AMENDED CLAIMS

[received by the International Bureau on 14 January 2004 (14.01.04);
original claims 1-464 replaced by amended claims 1-154]

1. A method for making minicells, comprising

(a) culturing a minicell-producing parent cell, wherein said parent cell comprises an expression construct, wherein said expression construct comprises a gene operably linked to expression sequences that are inducible and/or repressible, and wherein induction or repression of said gene causes or enhances the production of minicells; and

(b) separating said minicells from said parent cell, thereby generating a composition comprising minicells,

wherein an inducer or repressor is present within said parent cells during one or more steps and/or between two or more steps of said method.

2. The method of claim 1, further comprising

(c) purifying said minicells from said composition.

3. The method of claim 1, wherein said minicell is a eubacterial minicell.

4. The method of claim 1, wherein said gene expresses a gene product that is a factor that is involved in or modulates DNA replication, cellular division, cellular partitioning, septation, transcription, translation, or protein folding.

5. The method of claim 1, wherein said minicells are separated from said parent cell by a process selected from the group consisting of centrifugation, ultracentrifugation, density gradation, immunoaffinity and immunoprecipitation.

6. The method of claim 1, wherein said minicell-producing parent cell comprises a mutation in a gene required for lipopolysaccharide synthesis.

7. The method of claim 2, further comprising preparing a denuded minicell from said minicell.

8. The method of claim 2, further comprising covalently or non-covalently linking one or more components of said minicell to a conjugated moiety.

9. A method of preparing a L-form minicell comprising:

(a) culturing an L-form eubacterium, wherein said eubacterium comprises one or more of the following:

(i) an expression element that comprises a gene operably linked to expression sequences that are inducible and/or repressible, wherein induction or repression of said gene regulates the copy number of an episomal expression construct;

(ii) a mutation in an endogenous gene, wherein said mutation regulates the copy number of an episomal expression construct.

(iii) an expression element that comprises a gene operably linked to expression sequences that are inducible and/or repressible, wherein induction or repression of said gene causes or enhances the production of minicells; and

(iv) a mutation in an endogenous gene, wherein said mutation causes or enhances minicell production.

(b) culturing said L-form minicell-producing parent cell in media under conditions wherein minicells are produced; and

(c) separating said minicells from said parent cell, thereby generating a composition comprising L-form minicells,

wherein an inducer or repressor is present within said minicells during one or more steps and/or between two or more steps of said method.

10. The method of claim 9, further comprising

(d) purifying said L-form minicells from said composition.

11. A minicell comprising an immunogenic compound, wherein said minicell displays a binding moiety, wherein said binding moiety is part of a fusion protein comprising a first polypeptide that comprises at least one transmembrane domain or at least one membrane anchoring domain and a second polypeptide that comprises a binding moiety.

12. A minicell comprising an immunogenic compound, wherein said minicell displays a binding moiety, wherein said binding moiety is selected from the group consisting of (a) a eukaryotic membrane protein; (b) an archeabacterial membrane protein; (c) an organellar membrane protein; and (d) a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide, wherein said second polypeptide is not derived from a eubacterial protein and is neither a His tag nor a glutathione-S-transferase polypeptide, and wherein said polypeptide comprises a binding moiety.

13. The minicell of claim 11, wherein said binding moiety is selected from the group consisting of an antibody, an antibody derivative, a receptor and an active site of a non-catalytic derivative of an enzyme.

14. The minicell of claim 11, wherein said binding moiety is a single-chain antibody.

15. The minicell of claim 11, wherein said binding moiety is directed to a ligand selected from the group consisting of an epitope displayed on a pathogen, an epitope displayed on an infected cell and an epitope displayed on a hyperproliferative cell.

16. The minicell of claim 11, wherein said immunogenic compound is selected from the group consisting of a polypeptide and a nucleic acid.

17. The minicell of claim 11, further comprising a first and second nucleic acid, wherein said first nucleic acid comprises eukaryotic expression sequences operably linked to a first ORF, and a second nucleic acid, wherein said second nucleic acid comprises eubacterial expression sequences operably linked to a second ORF.

18. The minicell of claim 17, wherein one of said ORFs encodes a protein that comprises said binding moiety.

19. The minicell of claim 17, wherein said eubacterial expression sequences are induced and/or derepressed when said binding moiety is in contact with a target cell.

20. The minicell of claim 17, wherein said eukaryotic expression sequences are induced and/or derepressed when said nucleic acid is in the cytoplasm of a eukaryotic cell.

21. The minicell of claim 17, wherein the protein encoded by said first ORF comprises eukaryotic secretion sequences and/or the protein encoded by said second ORF comprises eubacterial secretion sequences.

22. The minicell of claim 12, wherein said minicell is a eubacterial minicell.

23. The minicell of claim 12, further comprisinga nucleic acid having an expression construct comprising expression sequences operably linked to an ORF encoding a protein selected from the group consisting of (i) said eukaryotic membrane protein, (ii) said archeabacterial membrane protein, (iii) said organellar membrane protein; and (iv) said fusion protein.

24. The minicell of claim 23, wherein said nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF, wherein said ORF encodes a therapeutic polypeptide.

25. The minicell of claim 24, wherein said therapeutic polypeptide is a membrane polypeptide.

26. The minicell of claim 24, wherein said therapeutic polypeptide is a soluble polypeptide.

27. The minicell of claim 26, wherein said soluble polypeptide comprises a cellular secretion sequence.

28. The minicell of claim 24, wherein said expression sequences are inducible and/or repressible.

29. The minicell of claim 28, wherein said expression sequences are induced and/or derepressed when the binding moiety displayed by said minicell binds to its target compound.

30. The minicell of claim 12 further comprising anucleic acid having an expression construct comprising expression sequences operably linked to an ORF, wherein said ORF encodes a polypeptide having an amino acid sequence that facilitates cellular transfer of an immunogenic compound contained within or displayed by said minicell.

31 The minicell of claim 12 wherein the membrane of said minicell comprises a system for transferring an immunogenic compound from the interior of a minicell into the cytoplasm of said cell.

32. A method of delivering an immunogenic compound to a cell, wherein said cell displays a ligand specifically recognized by a binding moiety, comprising contacting said cell with a minicell that displays said binding moiety, wherein said minicell comprises said immunogenic compound, and wherein the contents of said minicell are delivered into said cell from a minicell bound to said cell.

33. The method of claim 32, wherein said minicell is a eubacterial minicell.

34. The method of claim 32, wherein said immunogenic compound is selected from the group consisting of a nucleic acid and a polypeptide.

35. The method of claim 32, wherein the membrane of said minicell comprises a system for transferring said immunogenic compound from the interior of a minicell into the cytoplasm of said cell.

36. The method of claim 32, wherein said minicell further comprises a first and second nucleic acid, wherein said first nucleic acid comprises eukaryotic expression sequences operably linked to a first ORF, and a second nucleic acid, wherein said second nucleic acid comprises eubacterial expression sequences operably linked to a second ORF.

37. The method of claim 36, wherein one of said ORFs encodes a protein that comprises said binding moiety.

38. The method of claim 36, wherein said eubacterial expression sequences are induced and/or derepressed when said binding moiety is in contact with a target cell.

39. The method of claim 36, wherein said eukaryotic expression sequences are induced and/or derepressed when said nucleic acid is in the cytoplasm of a eukaryotic cell.

40. The method of claim 36, wherein the protein encoded by said first ORF comprises eukaryotic secretion sequences and/or the protein encoded by said second ORF comprises eubacterial secretion sequences.

41. A minicell displaying a synthetic linking moiety, wherein said synthetic linking moiety is covalenty or non-covalently attached to a membrane component of said mincell.

42. The minicell of claim 41, wherein said minicell is a eubacterial minicell.

43. A sterically stabilized minicell comprising a displayed moiety that has a longer half-life in vivo than a wild-type minicell, wherein said displayed moiety is a hydrophilic polymer that comprises a PEG moiety, a carboxylic group of a polyalkylene glycol or PEG stearate.

44. A minicell having a membrane comprising an exogenous lipid, wherein a minicell comprising said exogenous lipid has a longer half-life in vivo than a minicell lacking said exogenous lipid, and wherein said minicell is a eubacterial minicell.

45. The minicell of claim 44, wherein said exogenous lipid is a derivitized lipid.

46. The minicell of claim 45, wherein said derivitized lipid is a phosphatidylethanolamine derivatized with a compound selected from the group consisting of PEG, DSPE-PEG, PEG stearate, PEG-derivatized phospholipids, and PEG ceramides.

47. The minicell of claim 44, wherein said exogenous lipid is not present in a wild-type membrane, or is present in a different proportion than is found in minicells comprising a wild-type membrane.

48. The minicell of claim 47, wherein said exogenous lipid is selected from the group consisting of ganglioside, sphingomyelin, monosialoganglioside GM1, galactocerebroside sulfate, 1,2-sn-dimyristoylphosphatidylcholine, phosphatidylinositol and cardiolipin.

49. The minicell of claim 41, wherein said linking moiety is non-covalently attached to said minicell.

50. The minicell of claim 49, wherein one of said linking moiety and said membrane component comprises biotin, and the other comprises avidin or streptavidin.

51. The minicell of claim 41, wherein said synthetic linking moiety is a cross-linker.

52. The minicell of claim 51, wherein said cross-linker is a bifunctional cross-linker.

53. A pharmaceutical composition comprising a minicell, wherein said minicell displays a membrane conjugate, wherein said membrane conjugate comprises a membrane component chemically linked to a conjugated compound.

54. The pharmaceutical composition of claim 53, wherein said minicell is a eubacterial minicell.

55. The pharmaceutical composition of claim 53, wherein said membrane protein is selected from the group consisting of a receptor, a channel protein, a cellular adhesion factor and an integrin.

56. The pharmaceutical composition of claim 53, wherein said pharmaceutical further comprises an adjuvant.

57. The pharmaceutical composition of claim 53, wherein said membrane component is a polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain, or a lipid that is part of a membrane.

58. The pharmaceutical composition of claim 53, wherein said conjugated compound is a polypeptide, and the chemical linkage between said membrane compound and said conjugated compound is not a peptide bond.

59. The pharmaceutical composition of claim 53, wherein said conjugated compound is a nucleic acid.

60. The pharmaceutical composition of claim 53, wherein said conjugated compound is an organic compound.

61. The pharmaceutical composition of claim 60, wherein said organic compound is selected from the group consisting of a narcotic, a toxin, a venom, a sphingolipid, and a soluble protein.

62. The pharmaceutical formulation of claim 53, wherein said membrane component comprises a polypeptide epitope displayed by a hyperproliferative cell.

63. The pharmaceutical formulation of claim 53, wherein said membrane component comprises an epitope displayed by a eukaryotic pathogen, an archeabacterial pathogen, a virus or an infected cell.

64. The pharmaceutical formulation of claim 53, wherein said membrane component comprises an epitope selected from the group consisting of an epitope displayed by a eukaryotic pathogen, an archeabacterial pathogen, a virus and an infected cell.

65. The pharmaceutical formulation of claim 53, wherein said membrane componentis a fusion protein, said fusion protein comprising (i) a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and (ii) a second polypeptide, wherein said second polypeptide is not derived from a eubacterial protein.

66. A method of making a pharmaceutical formulation comprising a minicell, wherein said minicell displays a membrane conjugate, wherein said membrane conjugate comprises a membrane component chemically linked to a conjugated compound.

67. The method of claim 66, wherein said method further comprises adding an adjuvant to said pharmaceutical formulation.

68. The method of claim 66, wherein said membrane component is a polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain, or a lipid that is part of a membrane.

69. The method of claim 66, wherein said conjugated compound is a polypeptide, and the chemical linkage between said membrane compound and said conjugated compound is not a peptide bond.

70. The method of claim 66, wherein said conjugated compound is a nucleic acid.

71. The method of claim 66, wherein said conjugated compound is an organic compound.

72. The method of claim 71, wherein said organic compound is selected from the group consisting of a narcotic, a toxin, a venom, and a sphingolipid.

73. The method of claim 66, wherein said method further comprises adding an adjuvant to said pharmaceutical formulation.

74. The method of claim 66, wherein said method further comprises desiccating said pharmaceutical formulation.

75. The method of claim 66 wherein said method further comprises adding a suspension buffer to said pharmaceutical formulation.

76. The method of claim 66, wherein said method further comprises making a chemical modification of said membrane protein.

77. The method of claim 76, wherein said chemical modification is selected from the group consisting of glycosylation, deglycosylation, phosphorylation, dephosphorylation and proteolysis.

78. The method of making a pharmaceutical composition of claim 66, wherein said membrane component is a fusion protein, said fusion protein comprising (i) a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and (ii) a second polypeptide, wherein said second polypeptide is not derived from a eubacterial protein.

79. A method of making a antibody that specifically binds a protein domain, wherein said domain is in its native conformation, wherein said domain is contained within a protein displayed on a minicell, comprising contacting said minicell with a cell, wherein said cell is competent for producing antibodies to an antigen contacted with said cell, in order to generate an immunogenic response in which said cell produces said antibody.

80. The method of claim 79, wherein said minicell is a eubacterial minicell.

81. The method of claim 79, wherein said protein displayed on a minicell is a membrane protein.

82. The method of claim 81, wherein said membrane protein is a receptor or a channel protein.

83. The method of claim 79, wherein said domain is found within the second polypeptide of a membrane fusion protein, wherein said membrane fusion protein comprises a first polypeptide, wherein said first polypeptide comprises at least one transmembrane domain or at least one membrane anchoring domain.

84. The method of claim 79, wherein said antibody is a polyclonal antibody or a monoclonal antibody.

85. A method of making an antibody or antibody derivative that specifically binds an epitope, wherein said epitope is selected from the group consisting of (i) an epitope composed of amino acids found within a membrane protein, (ii) an epitope present in an interface between a membrane protein and a membrane component, (iii) an epitope present in an interface between a membrane protein and one or more other proteins and (iv) an epitope in a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain, and a second polypeptide, said second polypeptide comprising said epitope; comprising contacting a minicell displaying said epitope with a cell, wherein said cell is competent for producing antibodies to an antigen contacted with said cell, in order to generate an immunogenic response in which said cell produces said antibody.

86. The method of claim 85, wherein said minicell is a eubacterial minicell.

87. The method of claim 85, wherein said antibody is a polyclonal antibody.

88. The method of claim 85, wherein said antibody is a monoclonal antibody.

89. A method of determining the rate of transfer of nucleic acid from a minicell to a cell, comprising

(a) contacting said cell to said minicell, wherein said minicell comprises said nucleic acid, for a set period of time;

(b) separating said minicell from said cell;

(c) measuring the amount of nucleic acid in said cell,

wherein the amount of nucleic acid in said cell over said set period of time is the rate of transfer of a nucleic acid from a minicell.

90. A method of determining the amount of a nucleic acid transferred to a cell from a minicell, comprising

(a) contacting said cell to said minicell, wherein said minicell comprises an expression element having eukaryotic expression sequences operably linked to an ORF encoding a detectable polypeptide, wherein said minicell displays a binding moiety, and wherein said binding moiety binds an epitope of said cell; and

(b) detecting a signal from said detectable polypeptide,

wherein a change in said signal corresponds to an increase in the amount of said nucleic acid transferred to said cell.

91. The method of claim 89, wherein said minicell is a eubacterial minicell.

92. The method of claim 89, wherein said cell is a eukaryotic cell.

93. The method of claim 90, wherein said binding moiety is an antibody or antibody derivative.

94. The method of claim 90, wherein said binding moiety is a single-chain antibody.

95. The method of claim 90, wherein said binding moiety is an aptamer.

96. The method of claim 90, wherein said binding moiety is an organic compound.

97. The method of claim 90, wherein said detectable polypeptide is a fluorescent polypeptide.

98. A method of detecting the expression of an expression element in a cell, comprising

(a) contacting said cell to a minicell, wherein said minicell comprises an expression element having cellular expression sequences operably linked to an ORF encoding a detectable polypeptide, wherein said minicell displays a binding moiety, and wherein said binding moiety binds an epitope of said cell;

(b) incubating said cell and said minicell for a period of time effective for transfer of nucleic acid from said minicell to said cell; and

(c) detecting a signal from said detectable polypeptide,

wherein an increase in said signal corresponds to an increase in the expression of said expression element.

99. The method of claim 98, wherein said minicell is a eubacterial minicell.

100. The method of claim 98, wherein said cell is a eukaryotic cell and said expression sequences are eukaryotic expression sequences.

101. The method of claim 100, wherein said eukaryotic cell is a mammalian cell.

102. The method of claim 98, wherein said binding moiety is an antibody or antibody derivative.

103. The method of claim 98, wherein said binding moiety is a single-chain antibody.

104. The method of claim 98, wherein said binding moiety is an aptamer.

105. The method of claim 98, wherein said binding moiety is an organic compound.

106. The method of claim 98, wherein said detectable polypeptide is a fluorescent polypeptide.

107. A method for detecting the transfer of a fusion protein from the cytosol to an organelle of a eukaryotic cell, comprising

(a) contacting said cell to a minicell, wherein

(i) said minicell comprises an expression element having eukaryotic expression sequences operably linked to an ORF encoding a fusion protein, wherein said fusion protein comprises a first polypeptide that comprises organellar delivery sequences, and a second polypeptide that comprises a detectable polypeptide; and

(ii) said minicell displays a binding moiety that binds an epitope of said cell, or an epitope of an organelle;

(b) incubating said cell and said minicell for a period of time effective for transfer of nucleic acid from said minicell to said cell and production of said fusion protein; and

(c) detecting a signal from the detectable polypeptide,

wherein a change in the signal corresponds to an increase in the amount of the fusion protein transferred to said organelle.

108. The method of claim 107, wherein said organelle is a mitochondrion or a kinetoplast.

109. A method of introducing a nucleic acid into a cell, comprising contacting said cell with a minicell that comprises said nucleic acid, wherein said minicell displays a binding

moiety, wherein said binding moiety is selected from the group consisting of (i) a eukaryotic membrane protein; (ii) an archeabacterial membrane protein; (iii) an organellar membrane protein; and (iv) a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide, wherein said second polypeptide is not derived from a eubacterial protein and is neither a His tag nor a glutathione-S-transferase polypeptide, and wherein said polypeptide comprises a binding moiety; and wherein said binding moiety binds an epitope of said cell.

110. The method of claim 109, wherein said minicell is a eubacterial minicell.

111. The method of claim 109, wherein said nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF encoding a protein selected from the group consisting of (i) said eukaryotic membrane protein, (ii) said archeabacterial membrane protein, (iii) said organellar membrane protein; and (iv) said fusion protein.

112. The method of claim 109, wherein said nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF, wherein said ORF encodes a therapeutic polypeptide.

113. The method of claim 112, wherein said expression sequences are inducible and/or derepressible.

114. The method of claim 113, wherein said expression sequences are induced or derepressed when the binding moiety displayed by said minicell binds its target compound.

115. The method of claim 113, wherein said expression sequences are induced or derepressed by a transactivation or transrepression event.

116. The method of claim 111, wherein said nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF, wherein said ORF encodes a polypeptide having an amino acid sequence that facilitates cellular transfer of an immunogenic compound contained within or displayed by said minicell.

117. A minicell comprising a nucleic acid, wherein said nucleic acid comprises eukaryotic expression sequences and eubacterial expression sequences, each of which is independently operably linked to an ORF.

118. The minicell of claim 117, wherein said minicell is a eubacterial minicell.

119. The minicell of claim 117, wherein said minicell displays a binding moiety.

120. The minicell of claim 119, wherein said eubacterial expression sequences are induced and/or derepressed when said binding moiety is in contact with a target cell.

121. The minicell of claim 119, wherein said eukaryotic expression sequences are induced and/or derepressed when said nucleic acid is in the cytoplasm of a eukaryotic cell.

122. The minicell of claim 120, wherein the protein encoded by said ORF comprises eubacterial or eukaryotic secretion sequences.

123. A minicell comprising a first and second nucleic acid, wherein said first nucleic acid comprises eukaryotic expression sequences operably linked to a first ORF, and a second nucleic acid, wherein said second nucleic acid comprises eubacterial expression sequences operably linked to a second ORF.

124. The minicell of claim 123, wherein said minicell isa eubacterial minicell.

125. The minicell of claim 123, wherein said minicell displays a binding moiety.

126. The minicell of claim 125, wherein said eubacterial expression sequences are induced and/or derepressed when said binding moiety is in contact with a target cell.

127. The minicell of claim 125, wherein said eukaryotic expression sequences are induced and/or derepressed when said nucleic acid is in the cytoplasm of a eukaryotic cell.

128. The minicell of claim 123, wherein the protein encoded by said first ORF comprises eukaryotic secretion sequences and/or the protein encoded by said second ORF comprises eubacterial secretion sequences.

129. A method of introducing into and expressing a nucleic acid in an organism, comprising contacting a minicell to a cell of said organism, wherein said minicell comprises said nucleic acid.

130. The method of claim 129, wherein said minicell is a eubacterial minicell.

131. The method of claim 129, wherein said minicell displays a binding moiety.

132. The method of claim 129, wherein said nucleic acid comprises a eukaryotic expression construct, wherein said eukaryotic expression construct comprises eukaryotic expression sequences operably linked to an ORF.

133. The method of claim 129, wherein said ORF encodes a protein selected from the group consisting of a membrane protein, a soluble protein and a protein comprising eukaryotic secretion signal sequences.

134. The method of claim 129, wherein said nucleic acid comprises a eubacterial expression construct, wherein said eubacterial expression construct comprises eubacterial expression sequences operably linked to an ORF.

135. The method of claim 134, wherein said minicell displays a binding moiety, wherein said eubacterial expression sequences are induced and/or derepressed when said binding moiety is in contact with a target cell.

136. The method of claim 135, wherein the protein encoded by said ORF comprises eubacterial secretion sequences.

137. A minicell-producing parent cell, wherein said parent cell comprises one or more of the following:

(a) an expression element that comprises a gene operably linked to expression sequences that are inducible and/or repressible, wherein induction or repression of said gene regulates the copy number of an episomal expression construct;

(b) a mutation in an endogenous gene, wherein said mutation regulates the copy number of an episomal expression construct;

(c) an expression element that comprises a gene operably linked to expression sequences that are inducible and/or repressible, wherein induction or repression of said gene causes or enhances the production of minicells; and

(d) a mutation in an endogenous gene, wherein said mutation causes or enhances minicell production.

138. The minicell-producing parent cell of claim 137, further comprising an episomal expression construct.

139. The minicell-producing parent cell of claim 137, further comprising a chromosomal expression construct.

140. The minicell-producing parent cell of claim 137, wherein said minicell-producing parent cell comprises an immunogenic compound.

141. The minicell of claim 137 wherein said gene that causes or enhances the production of minicells has a gene product that is involved in or regulates DNA replication, cellular division, cellular partitioning, septation, transcription, translation, or protein folding.

142. A minicell-producing parent cell, wherein said parent cell comprises an expression construct, wherein said expression construct comprises expression sequences operably linked to an ORF that encodes a protein, and a regulatory expression element, wherein said regulatory expression element comprises expression sequences operably linked to a regulatory gene that encodes a factor that regulates the expression of said ORF.

143. The minicell-producing parent cell of claim 142, wherein said expression sequences of said expression construct are inducible and/or repressible.

144. The minicell-producing parent cell of claim 142, wherein said expression sequences of said regulatory expression construct are inducible and/or repressible.

145. The minicell-producing parent cell of claim 142, wherein one or more of said expression element or said regulatory expression element is located on a chromosome of said parent cell.

146. The minicell-producing parent cell of claim 142, wherein one or more of said expression element or said regulatory expression element is located on an episomal expression construct.

147. The minicell-producing parent cell of claim 146, wherein both of said expression element and said regulatory expression element are located on an episomal expression construct, and one or both of said expression element and said regulatory expression element segregates into minicells produced from said parent cell.

148. The minicell-producing parent cell of claim 142, wherein said minicell-producing parent cell comprises an immunogenic compound.

149. The minicell-producing parent cell of claim 148, wherein said immunogenic compound segregates into minicells produced from said parent cell.

150. The minicell-producing parent cell of claim 142, wherein said ORF encodes a membrane protein or a soluble protein.

151. The minicell-producing parent cell of claim 142, wherein said protein comprises secretion sequences.

152. The minicell-producing parent cell of claim 142, wherein said factor is a transcription factor.

153. The minicell-producing parent cell of claim 148, wherein said factor is a RNA polymerase.

154. The minicell-producing parent cell of claim 153, wherein said parent cell is MC-T7.